

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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| In re Application of                      | ) |                       |
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| Ludviksson <i>et al.</i>                  | ) |                       |
|   | ) | Group Art Unit: 1644  |
| Serial No. 09/856,544                     | ) |                       |
|   | ) | Examiner: Haddad, M.  |
| Filed: December 20, 2001                  | ) |                       |
|   | ) | Confirmation No. 9811 |
| FOR: ANTAGONISTS OF THE $\alpha E\beta 7$ | ) |                       |
| INTEGRIN AS THERAPEUTIC AGENTS FOR        | ) |                       |
| INFLAMMATORY DISEASES                     | ) |                       |

DECLARATION OF EUGENE C. BUTCHER UNDER 37 C.F.R. § 1.132

Commissioner for  
Patents  
Washington, D.C. 20231

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Sir:

I, Eugene C. Butcher, a citizen of the United States of America, residing at 230 Corte Madera, Portola Valley, CA 94028, declare that:

1. I have read and understood the above-referenced patent application.
2. I received my M.D. degree from Washington University in St. Louis, Missouri. I have been conducting research in the field of inflammation since 1978 and am a co-author of over 200 publications relating to inflammation. I am currently a Professor in the Department of Pathology at Stanford University and a co-founder of Bioseek, Inc.
3. It is my belief that although U.S. Patent No. 5,610,281 (the '281 patent) discloses the administration of an isolated peptide derived from the extracellular domain of E-cadherin, which

binds to  $\alpha E\beta 7$  and inhibits the adhesion between an IEL and E-cadherin, this interaction between  $\alpha E\beta 7$  and E-cadherin is not relevant to the present claims, because E-cadherin is expressed in epithelial cells and is not expressed to any appreciable extent in non-epithelial cells of the gut, such as the lamina propria, where inflammatory bowel disease manifests itself (Strauch et al. "Integrin  $\alpha E\beta 7$  Mediates Adhesion to Intestinal Microvascular Endothelial Cell Lines Via an E-Cadherin-Independent Interaction" *J. Immunol.* 166: 3506-3514, 2001; Hanby et al. "Downregulation of E-Cadherin in the Reparative Epithelium of the Human Gastrointestinal Tract" *Am. J. Pathol.* 148:723-9, 1996; Cepek et al. "Integrin  $\alpha E\beta 7$  mediates adhesion of T-lymphocytes to epithelial cells" *J. Immunol.* 150:3459, 1993; Cepek et al. "Adhesion between epithelial cells and T lymphocytes mediated by E-cadherin and the  $\alpha E\beta 7$  integrin" *Nature* 372:190, 1994; Karcela et al. "Recognition of E-cadherin on epithelial cells by the mucosal T cell integrin  $\alpha M290\beta 7$  ( $\alpha E\beta 7$ )" *Eur. J. Immunol.* 25:852, 1995; Higgins et al. "Direct and regulated interaction of integrin  $\alpha E\beta 7$  with E-cadherin" *J. Cell Biol.* 140:197, 1998). Therefore, the suggestion that the interaction between  $\alpha E\beta 7$  and E-cadherin can be inhibited to treat inflammatory bowel disease is not supported by the longstanding understanding of E-cadherin expression and mechanisms of inflammatory bowel disease. My belief is based on the following additional scientific facts and observations.

Although E-cadherin has been identified as an  $\alpha E\beta 7$  ligand, this is not the only ligand for  $\alpha E\beta 7$ .  $\alpha E\beta 7$ , like other integrins, binds to more than one ligand. For example, the I-domain containing integrin,  $\alpha Mb2$ (Mac 1), binds a wide repertoire of ligands, including ICAM-1, fibrinogen, iC3b, factor X, denatured proteins, neutrophil inhibitory factor, lipopolysaccharide and zymosan. Yalamanchili et al. (*J. Biol. Chem.* 275: 21377, 2000) have shown that the I-domain is required for binding to some ligands and not others. Similarly, another integrin,  $\alpha 4\beta 7$ , has multiple ligands, including MadCAM-1, VCAM-1 and fibronectin. Monoclonal antibodies have been generated that define unique patterns of inhibition for  $\alpha 4\beta 7$  binding to each of its defined molecular ligands (Andrew DP, *J. Immunol.* 153:3847, 1994). Yet another example is the  $\alpha 3\beta 1$  integrin which binds collagen, laminin and fibronectin. However, only the binding of  $\alpha 3\beta 1$  integrin to fibronectin, not the binding of  $\alpha 3\beta 1$  integrin to laminin or collagen, can be

inhibited by RGD-containing peptides (Elices, MJ, *J. Cell Biol.* 112:169, 1991). The  $\alpha E\beta 7$  integrin is distinct from other integrins in containing both an I-domain and an X domain of 55 amino acids. Binding of  $\alpha E$  to E-cadherin does not appear to involve the X domain and it is believed that the X domain may be involved in interactions with yet unidentified  $\alpha E\beta 7$  ligands (Higgins et al. *J. Biol. Chem.* 275:25662, 2000). Therefore, it is well accepted that an inhibitor of a ligand for a particular integrin is not expected to be an inhibitor of all of the ligands which bind to that integrin.

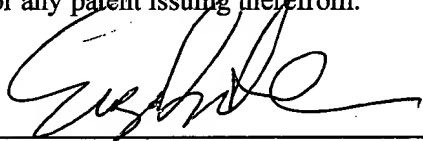
As stated above, it is also known that E-cadherin is not expressed to any appreciable extent in the lamina propria of the gut. Therefore, it is highly unlikely that inflammatory bowel disease is mediated via an  $\alpha E\beta 7$ -E-cadherin interaction. The publication by Strauch et al. ("Integrin alpha E(CD103)beta 7 mediates adhesion to intestinal microvascular endothelial cell lines (HIMEC) via an E-cadherin-independent interaction," *J. Immun.* 166: 3506-3514, 2001) reports that  $\alpha E\beta 7$  mediates adhesion to intestinal endothelial cells in the lamina propria *in vivo*, via an E-cadherin-independent interaction. Strauch et. al. also suggest the presence of a second ligand for  $\alpha E\beta 7$  that is clearly distinct from E-cadherin and with a different expression pattern. This reference also showed that a mAb that blocks E-cadherin mediated cell binding to  $\alpha E\beta 7$ , did not block binding of HIMEC to an  $\alpha E\beta 7$ -Fc. Furthermore, HIMEC did not express detectable levels of E-cadherin.

Another reference has suggested that  $\alpha E\beta 7$  mediates lymphocyte binding to a skin-derived epithelial cell line through an E-cadherin independent interaction (Brown et al. "Mechanisms of binding of cutaneous lymphocyte-associated antigen-positive and  $\alpha E\beta 7$ -positive lymphocytes to oral and skin keratinocytes" *Immunology* 98:9-15, 1999). Further evidence that  $\alpha E\beta 7$  can have a different, E-cadherin independent role in cell homing and inflammation comes from gene expression analysis showing that  $\alpha E\beta 7$  is expressed on T cell populations outside the intraepithelial area, e.g. in areas that do not contain E-cadherin expressing cells (Gavin M. et al., "Homeostasis and anergy of CD4(+)CD25(+) suppressor T cells *in vivo*" *Nat. Immunol.*, 3(1):33, 2002; Zelenika D., "Regulatory T cells overexpress a subset of Th2 gene transcripts" *J. Immunol.* 168(3): 1069, 2002; McHugh RS et al.,

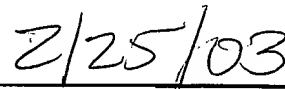
"CD4(+)CD25(+) immunoregulatory T cells: gene expression analysis reveals a functional role for the glucocorticoid-induced TNF receptor" *Immunity* 16(2):311, 2002).

Therefore, it would not be expected that the identification of an inhibitor of E-cadherin binding to  $\alpha E\beta 7$ , would provide an inhibitor for the interaction of  $\alpha E\beta 7$  with another ligand, such as the ligand involved in inflammatory bowel disease. Although the '281 patent discloses that administration of an E-cadherin peptide leads to decreased localization of intra-epithelial lymphocytes to the intraepithelial compartment, the '281 patent does not show or suggest that an  $\alpha E\beta 7$  Mab will inhibit binding of  $\alpha E\beta 7$  to another ligand or that an  $\alpha E\beta 7$  Mab will inhibit any activity associated with the interaction of  $\alpha E\beta 7$  with a non-E cadherin ligand. Furthermore, because E-cadherin is not found at appreciable levels in the lamina propria, it would not be likely that an E-cadherin based mechanism would be successful for treatment of inflammatory bowel disease, and it would not have been obvious, at the time the present invention was made, to target an E-cadherin ligand, e.g.  $\alpha E\beta 7$ , to treat inflammatory bowel disease.

4. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or document or any patent issuing therefrom.



EUGENE C. BUTCHER



DATE